Effects of PSK(Krestin) on Protection of *Pseudomonas aeruginosa* Infection and Healing of Burn Wound in Burned Mice

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Summary

The protective effects of PSK(Krestin) salve consisting of Solbase and PSK on *Pseudomonas aeruginosa* burn wound infection induced in mice were studied.

PSK salve enhanced clearance of *p. aeruginosa* from infected burn wounds and also accelerated the healing in mice. Elimination of *p. aeruginosa* and healing of burn wounds depended on the number of applications of PSK salve.

Among opportunistic pathogens, *Pseudomonas aeruginosa* is strongly associated with severe infections after burn injury(1, 14), because the host defense mechanism of burned animals is markedly altered by burn(8, 10). On the other hand, it is proved that PSK (protein-bound polysaccharide preparation from *Coriolus versicolor*) which are currently prescribed for cancer immunotherapy(3, 9, 13, 16) also enhances the resistance to experimental bacterial(15) and viral(5, 6) infections in animals.

There are no reports on protective effects of PSK against *Pseudomonas aeruginose* infection in burned models. This paper deals with the bacteria-eliminating and burn wound-healing effects of PSK on *Pseudomonas aeruginosa* burn wound infection induced in mice.

PSK was donated by Kureha Chemical Ind., Co., Tokyo. Five-week-old female ddy mice purchased from Seiwa Experimental Animal Co., Fukuoka. Burned mice were prepared as follows. In mice anesthetized with pentobarbital(about 60 mg/kg), a three-sec burn was given to clipped dorsum by an iron trowel(2×3 cm) heated over a

Bunsen burner flame. Approximately 10% of the total body surface was burned in the third degree. Twenty-four hr later, the necrotic skin was removed and 0.5g PSK salve consisting of 0.1ml PSK(1 and 10 mg/ml) and 0.4 g Solbase (Dai-Nippon Pharmaceutical Co., Osaka) was spread on the burned area of mice.

Twenty-four hr after application of the PSK salve, the burned area was infected with 0.1ml of 10^9/ml of suspension of *p. aeruginosa* PAO 3047 in saline. Every seven days, the infected area was wiped with a sterile wet cotton swab and the swab was immersed and shaken in saline(2 ml) to free the attached organisms. The colony-forming units(CFUs) per cm^2 of burned area were determined on a nalidixic acid-setrimide(NAC) agar plate.

As shown in Fig. 1, the number of organisms recovered from the burned area of control animals 7, 14, 21 and 28 days after infection were $3.6\pm0.4\times10^6$, $4.2\pm0.1\times10^5$, $5.3\pm0.7\times10^4$ and $2.1\pm0.5\times10^3$, respectively. On the ther hand, the numder of CFUs recovered from the infected burned area pretreated with 0.1 mg PSK salve was much the same as that from controls. However, the number of CFUs was significantly($p<0.05$) reduced by the application of 1 mg PSK salve: CFUs at 7, 14, 21 and 28 days after infection were $7.3\pm1.4\times10^5$, $3.9\pm0.6\times10^4$, $3.5\pm1.1\times10^3$ and $5.0\pm0.4\times10^2$, respectively. Further studies were done on the correlation between the number of CFUs and application times of PSK salve. The wound was treated three times with PSK (1 mg) salve at 24 hr-intervals, and infected with *p. aeruginosa*(10^8) 24 hr after the last treatment with PSK salve. As shown in Fig. 2, the decrease in the number of CFUs depended on the number of applications of the PSK salve.

In general, the healing mechanism of burn wound is due to the accumulation of phagocytic cells and/or fibroblasts, and consequently the formation of collagen fibers in sucutaneous tissues of burn injury. we also studied whether or not PSK salve accelerates the healing of burn wound infected with *p. aeruginosa*. Every seven days, the shape of the burned site was transcrived onto a tracing paper and the area was measured with a modulator system(Type: MOP/AM3, Kontron Co., W. Germany). As shown in Fig. 3, the burned area of control animals 14, 21 and 28 days after infection was 4.2 ± 0.9, 2.2 ± 0.8 and 1.0 ± 0.2 cm^2, respectively. When the burned area was treated once with PSK(1 mg) salve, there was no significant differences in the burned area between the control and the experimental groups. In contrast, the burned area of animals treated three times with PSK salve 14, 21 and 28 days after infection was smaller than that of controls, that is, 2.5 ± 0.4, 0.9 ± 0.2 and 0.4 ± 0.1 cm^2, respectively ($p<0.05$). It was also examined the histopathological changes in subcutaneous tissues of burn wound 21 days after infection. The lesions of animals pretreated with PSK

salve were mainly consisted of collagen fibers, stained red or rose-color by Elastica-Van Giesen stain, whereas the degree of the formation of collagen fibers in the lesions of subcutaneous tissues in control animals was inferior to that of PSK salve-treated animals (figures not shown). The periods for complete healing of burn wound in control animals were required for 39.7 ± 1.2 days, while the periods were significantly shortened by the treatment with PSK salve (35.9 ± 1.2, $p. < 0.05$).

A slight burn has little effect on the host immune system and the host nutritious condition, while with an extensive deep burn wound, the nutriture of host is aggravated, and consequently the host defense mechanism is impaired (1, 8, 10, 14). Therefore, burned skin becomes susceptible to infections(11, 14). Systemic and topical administration of antibiotics decreased the number of cases of *Pseudomonas* septicemia, a principal causes of death related to burn injury(4). In the case of the extensive third-degree burns, antibiotics administered systemically have few beneficial effects as the blood supply to the burned area is diminished. Application of topical antimicrobial agents is more effective than systemic antimicrobial therapy(11). As topical antimicrobial agents, 0.5% silver nitrate(12), 10% Sulfamylon cream(7) and Silvadene(Geben) cream(2) are widely used.

In the present study, it became evident that PSK salve enhances the clearance of *p. aeruginosa* from infected burn wounds and accelerates the healing of burn injury in burned mice. The results obtained may be due to the rapid accumulation of phagocytic cells and proliferation of fibroblasts by PSK. It is suggested the possibility that the clinical use of PSK salve for burn wounds is effective to prevent bacterial infections or to control the bacterial proliferation and hence accelerate the healing of burn wound. However, it is also required for restoration of nutritious conditions in patients.

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Legends

FIG. 1. Changes in the number of *p. aeruginosa* in the burn wound following application of PSK salve.

A burn was given to the clipped dorsum of mice and 0.5 g PSK salve consisting of Solbase (0.5 g) and PSK(0.1 or 1 mg) was spread over the wound. Twenty-four hrlater, *p. aeruginosa* PAO 3047(10^8) was applied. Every 7 days, the infected area was swabbed, and CFUs per cm^2 of burned area were determined on a NAC agar plate. Control(○), PSK(0.1 mg) salve(▲), PSK(1 mg) salve(●). *p* values were calculated by Student's *t*-test. Bar: mean value±S.E. (n=5), $^{※}$ $p<0.05$

FIG. 2. Administration-times of PSK salve and the number of *p. aeruginosa* in the burn wound

To the burned dorsum of mice was applied PSK(1 mg) salve three times at 24 hr intervals, and infected with *p. aeruginosa*(10^8) was given 24 hr after the third treatment. CFUs were assayed as described in the legend for figure 1. Application of PSK salve: none(○), once(■), twice(▲), 3 times(●). Bar: mean value ± S.E. (n=5),$^{※}$ $p<0.05$, $^{※※}p<0.01$

FIG. 3. Changes in the burned area by topical application of PSK salve

Burned mice were infected with *p. aeruginosa*(10^8) 24 hr after application of PSK(1 mg) salve three times at 24 hr intervals. Every seven days, the shape of the burn wound was transcribed onto tracing paper and the burned area was measured with a modulator system. Application of PSK salve: none(○), once(■), twice(▲), three times(●). Bar: mean value ± S.E. (n=5), $^{※}$ $p<0.05$

FIG. 1

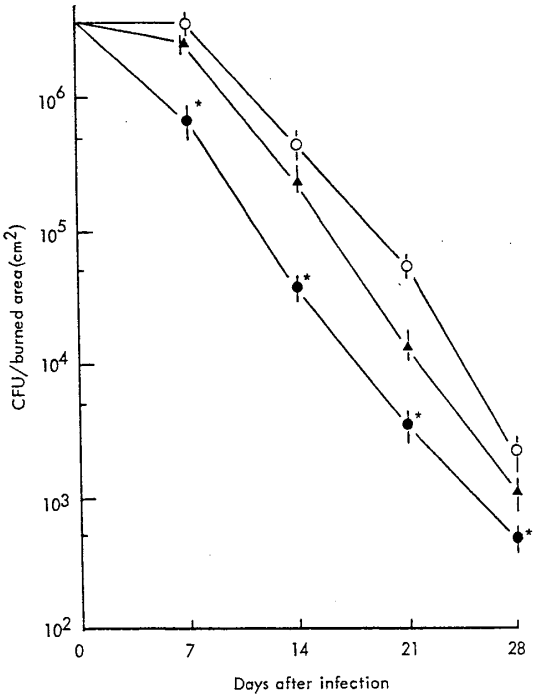

FIG. 2

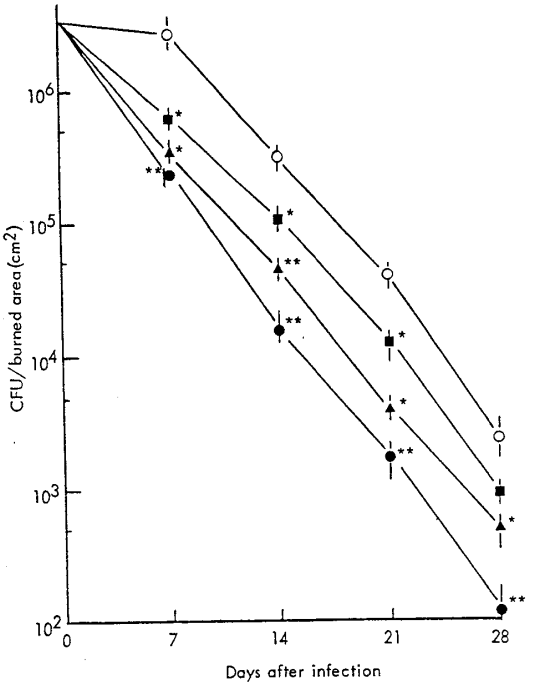

FIG. 3.